

### What is claimed is:

[Claim 1] 1. A method of quantifying a presence of a specific kind of probiotic microorganism in a sample of animal feed, said method comprising:

(a) culturing the sample under conditions suitable for growth of cultures of the specific kind of probiotic microorganism;

(b) using at least one oligonucleotide to detect the presence or absence of the specific kind of probiotic microorganism in respective portions of the cultured sample; and

(c) quantifying the presence of the specific kind of probiotic microorganism in the sample of material from the detected presence or absence of the specific kind of probiotic microorganism in the respective portions of the cultured sample.

[Claim 2] 2. The method as claimed in claim 1, which includes taking the sample of animal feed from a feedpile and transporting the sample to a testing lab in such a way that the sample of the animal feed at the testing laboratory is representative of the condition of the animal feed when the animal feed is to be consumed by animals.

[Claim 3] 3. The method as claimed in claim 1, which includes taking the sample of animal feed from a feedpile at a location where the animal feed is to be consumed by animals.

[Claim 4] 4. The method as claimed in claim 1, wherein the specific kind of probiotic microorganism is a species of *Lactobacillus*.

[Claim 5] 5. The method as claimed in claim 1, wherein the specific kind of probiotic microorganism is *Lactobacillus acidophilus*.

[Claim 6] 6. The method as claimed in claim 1, wherein the specific kind of probiotic microorganism is *Lactobacillus LA-51*.

[Claim 7] 7. The method as claimed in claim 1, wherein said at least one oligonucleotide hybridizes with a nucleic acid sequence that is indicative of a species of the specific kind of microorganism.

[Claim 8] 8. The method of claim 1, wherein the sample is cultured on a plate of culture media, and the respective portions of the cultured sample are taken from respective colonies of microorganisms that have been found to have grown on the plate of culture media.

[Claim 9] 9. The method of claim 1, wherein the sample is cultured by dividing the sample into multiple portions and culturing each portion, and wherein the presence or absence of the specific kind of microorganism is detected in each cultured portion.

[Claim 10] 10. The method as claimed in claim 9, wherein the sample is divided into the multiple portions by diluting the sample and dividing the diluted sample into the multiple portions.

[Claim 11] 11. The method as claimed in claim 9, wherein the sample is divided into multiple portions by mixing the sample with liquid to produce a fluid mixture, and dividing the fluid mixture into the multiple portions.

[Claim 12] 12. The method as claimed in claim 1, wherein the using of at least one oligonucleotide to detect the presence or absence of the specific kind of probiotic microorganism in respective portions of the cultured sample includes detecting the presence or absence of a product of hybridization of

said at least one oligonucleotide with a nucleic acid sequence that is indicative of the specific kind of probiotic microorganism.

[Claim 13] 13. The method as claimed in claim 1, wherein the using of at least one oligonucleotide to detect the presence or absence of the specific kind of probiotic microorganism in respective portions of the cultured sample includes using two oligonucleotide primers that induce a polymerase chain reaction in the presence of nuclear material of the specific kind of probiotic microorganism, and detecting the presence or absence of a product of the polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of the specific kind of probiotic microorganism.

[Claim 14] 14. The method as claimed in claim 13, wherein one of the oligonucleotide primers hybridizes with a nucleic acid sequence indicative of the genus of the specific kind of microorganism, and another of the oligonucleotide primers hybridizes with a nucleic acid sequence indicative of the species of the specific kind of probiotic microorganism.

[Claim 15] 15. The method as claimed in claim 13, wherein the detecting of the presence or absence of a product of the polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of the specific kind of probiotic microorganism includes performing electrophoresis of polymerase chain reaction products to detect a reaction product having a characteristic molecular length indicative of a polymerase chain reaction of the two oligonucleotide primers in the presence of the nuclear material of the specific kind of probiotic microorganism.

[Claim 16] 16. The method as claimed in claim 1, wherein the presence of the specific kind of probiotic microorganism in the sample of material is quantified in terms of a most probable number of the specific kind of probiotic microorganism.

**[Claim 17]** 17. A method of quantifying a presence of a specific kind of probiotic microorganism in a sample of animal feed, said method comprising:

(a) dividing the sample into multiple portions;  
(b) culturing each portion of the sample under conditions suitable for growth of a culture of the specific kind of probiotic microorganism;

(c) performing a polymerase chain reaction process by reacting each cultured portion of the sample successively with two oligonucleotide primers that selectively hybridize with nucleic acid of the specific kind of probiotic microorganism to produce a respective reaction product from each cultured portion of the sample;

(d) detecting the presence or absence of a reaction product having a characteristic length from the reaction of each cultured portion of the sample;  
and

(e) quantifying the presence of the specific kind of probiotic microorganism in the sample of material from the detected presence or absence of a reaction product having a characteristic length from the reaction of each cultured portion of the sample.

**[Claim 18]** 18. The method as claimed in claim 17, wherein the presence of the specific kind of probiotic microorganism in the sample of material is quantified in terms of a most probable number of the specific kind of probiotic microorganism in the sample of material.

**[Claim 19]** 19. The method as claimed in claim 17, wherein the sample is diluted prior to the culturing of the portions of the sample so that a good number of the cultured portions of the sample have an absence of a reaction product having the characteristic length.

**[Claim 20]** 20. The method as claimed in claim 17, wherein the two oligonucleotide primers include one oligonucleotide primer that hybridizes

with a nucleic acid sequence indicative of a genus of the specific kind of probiotic microorganism, and another oligonucleotide primer that hybridizes with a nucleic acid sequence indicative of the species of the specific kind of probiotic microorganism.

[Claim 21] 21. The method as claimed in claim 17, wherein the specific kind of probiotic microorganism is a species of *Lactobacillus*.

[Claim 22] 22. The method as claimed in claim 17, wherein the specific kind of probiotic microorganism is *Lactobacillus acidophilus*.

[Claim 23] 23. The method as claimed in claim 17, wherein the specific kind of probiotic microorganism is *Lactobacillus LA-51*.

[Claim 24] 24. The method as claimed in claim 17, which includes taking the sample of animal feed from a feedpile at a location where the animal feed is to be consumed by animals.

[Claim 25] 25. A method for the detection of probiotic microorganisms in animal feed, the method comprising:

- contacting animal feed and a probiotic microorganism to produce a treated animal feed;

- obtaining a sample of treated animal feed;

- culturing the sample under conditions suitable for growth of the probiotic microorganism;

- performing a polymerase chain reaction (PCR) on the cultured sample using two PCR primers to produce a PCR product;

- analyzing the PCR product to obtain a PCR reaction result; and

- correlating the PCR reaction result with the presence or absence of the probiotic microorganism in the animal feed.

[Claim 26] 26. The method as claimed in claim 25, wherein the sample of treated animal feed is taken at a location where the treated animal feed is consumed by animals.

[Claim 27] 27. The method as claimed in claim 25, wherein the probiotic microorganism is a species of *Lactobacillus*.

[Claim 28] 28. The method as claimed in claim 25, wherein the probiotic microorganism is *Lactobacillus acidophilus*.

[Claim 29] 29. The method as claimed in claim 25, wherein the probiotic microorganism is *Lactobacillus LA-51*.

[Claim 30] 30. The method as claimed in claim 25, wherein the polymerase chain reaction is performed on multiple portions of the cultured sample, the PCR product from the multiple portions is analyzed to obtain respective reaction results the multiple portions of the cultured sample, and the respective reaction results from the multiple portions of the cultured sample are correlated to determine a most probable number of the probiotic microorganism in the sample of treated animal feed.

[Claim 31] 31. A method for the detection of probiotic microorganisms in animal feed, the method comprising:

- contacting animal feed and a probiotic microorganism to produce a treated animal feed;

- obtaining a sample of treated animal feed;

- culturing the sample under conditions suitable for growth of the probiotic microorganism to produce a culture;

- obtaining nucleic acid from the culture;

contacting the nucleic acid with an oligonucleotide under conditions suitable for formation of a hybridized oligonucleotide–nucleic acid;  
detecting the hybridized oligonucleotide–nucleic acid to obtain a hybridization result; and  
correlating the hybridization result with the presence or absence of the probiotic microorganism in the animal feed.

[Claim 32] 32. The method as claimed in claim 31, wherein the sample of treated animal feed is taken at a location where the treated animal feed is consumed by animals.

[Claim 33] 33. The method as claimed in claim 31, wherein the microorganism is a species of *Lactobacillus*.

[Claim 34] 34. The method as claimed in claim 31, wherein the specific kind of microorganism is *Lactobacillus acidophilus*.

[Claim 35] 35. The method as claimed in claim 31, wherein the specific kind of microorganism is *Lactobacillus LA-51*.

[Claim 36] 36. The method as claimed in claim 31, wherein multiple portions of the sample are cultured, respective hybridization results are obtained for the cultured portions of the sample, and the respective hybridization results are correlated to determine a most probable number of the microorganism in the sample of treated animal feed.